Revealing the urinary exposome of smokers, vapers, HTP users and pouch consumers by high-resolution LC-MS – an important step in the identification of use-specific biomarkers of exposure

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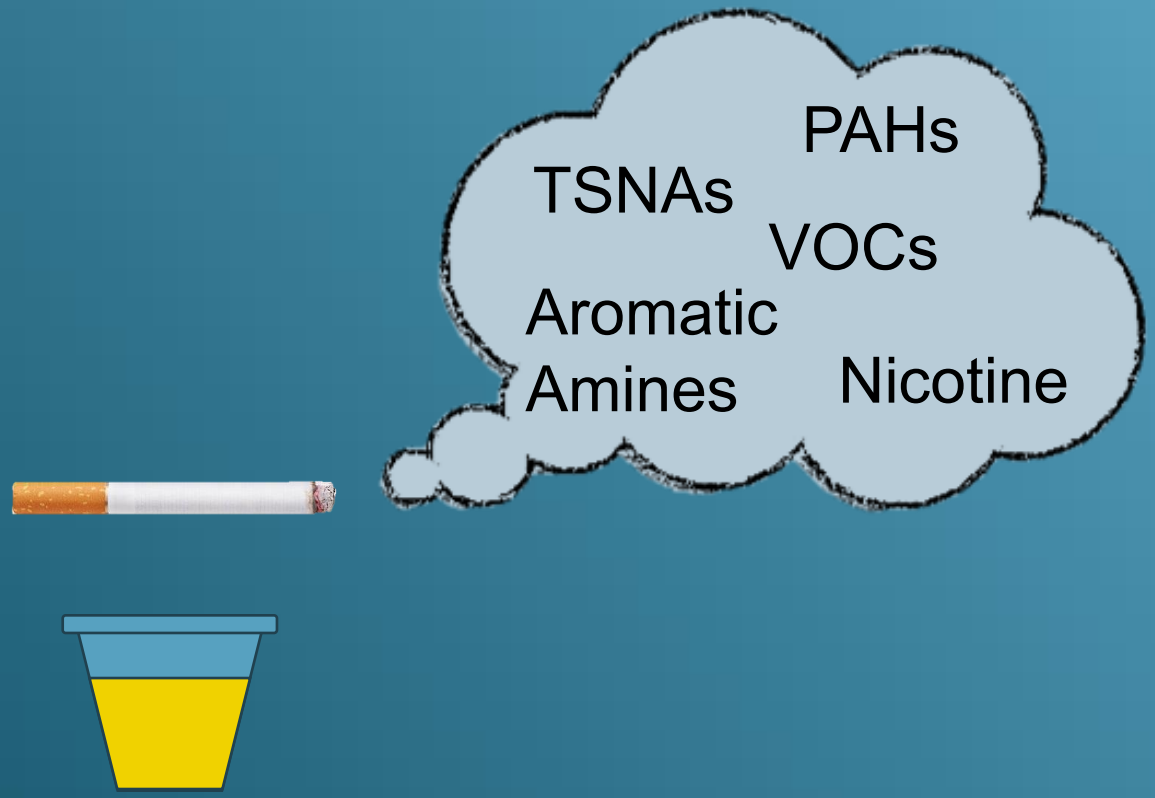
The Exposome

- Exposome: the totality of human environmental exposures[1]
- Numerous harmful&potentially harmful constituents (HPHCs) in tobacco smoke
- Current studies in tobacco harm reduction measure biomarkers of exposure (BoE) related to these HPHCs
- Targeted analysis of urinary metabolites of PAHs, VOCs, TSNAs, nicotine, …
- Problem: Those BoEs related to combustion and (some more, some less) specific to smoking combustible cigarettes

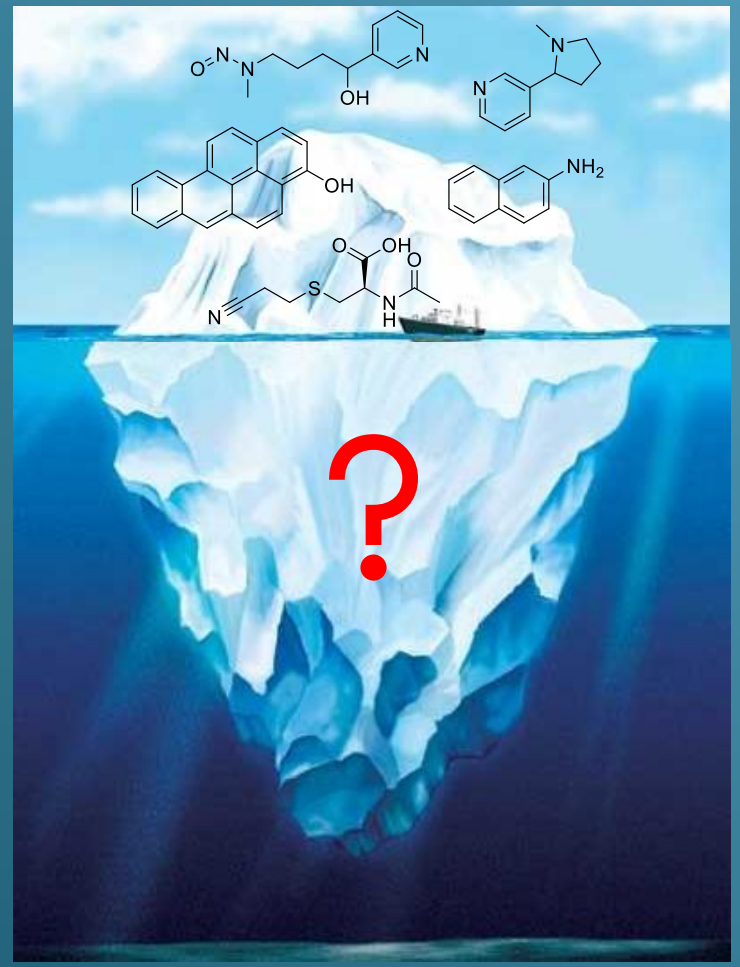


[1] Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 2005;14:1847–50.

The Exposome

Study objective

Decipher the urinary exposome to identify BoE which are capable of differentiating the consumers of different nicotine products

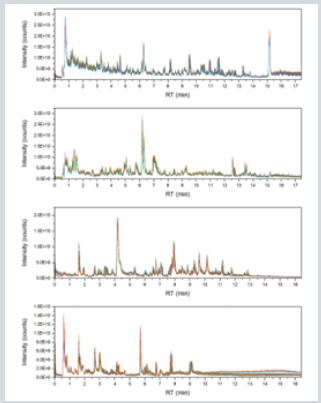


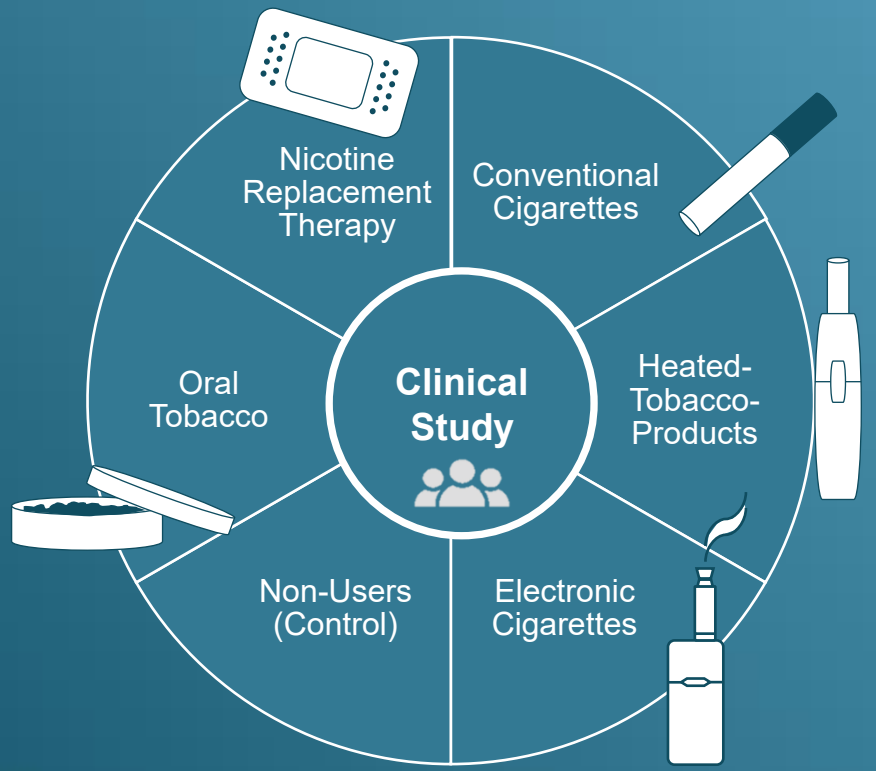
[1] Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 2005;14:1847–50.

Clinical Study

- Exclusive, sole users of 5 different nicotine product categories and non-users as control
- 3 days confinement (ad lib use, diet-controlled)
- Biospecimens: 24h-urine, blood, saliva, exhaled breath and exhaled breath condensate

Non-targeted analysis in 24h urine by LC-MS (Thermo Qexactive HF-X)



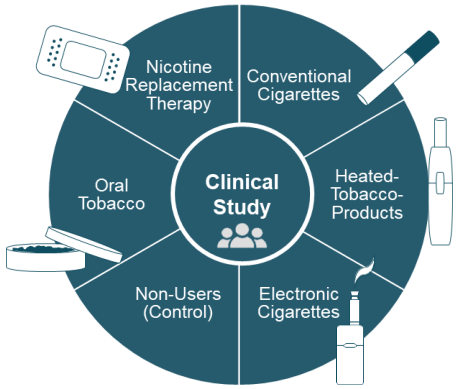


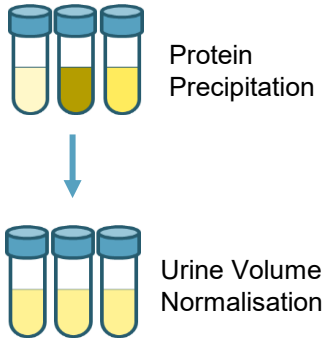
10 subjects per group

Day 1 → Day 2 → Day 3

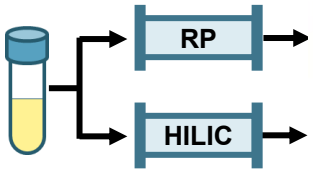
Sibul et al (2021): Identification of biomarkers specific to five different nicotine product user groups: Study protocol of a controlled clinical trial. *Contemporary Clinical Trials Communications*, 22

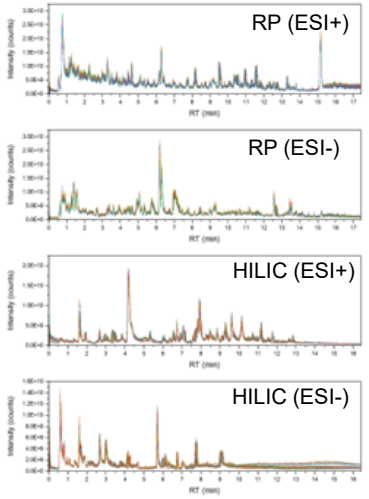
Non-targeted analysis – Workflow



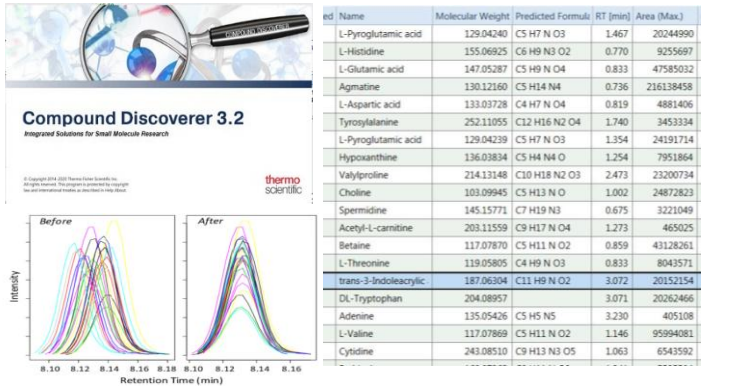


Sample processing

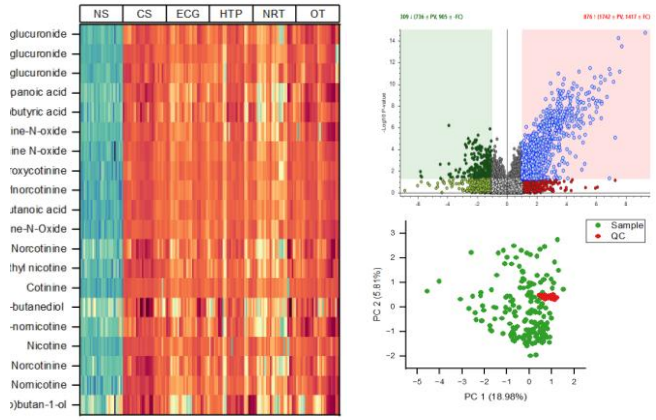




Data acquisition



Data pre-processing:
RT alignment / Intensity correction by QC



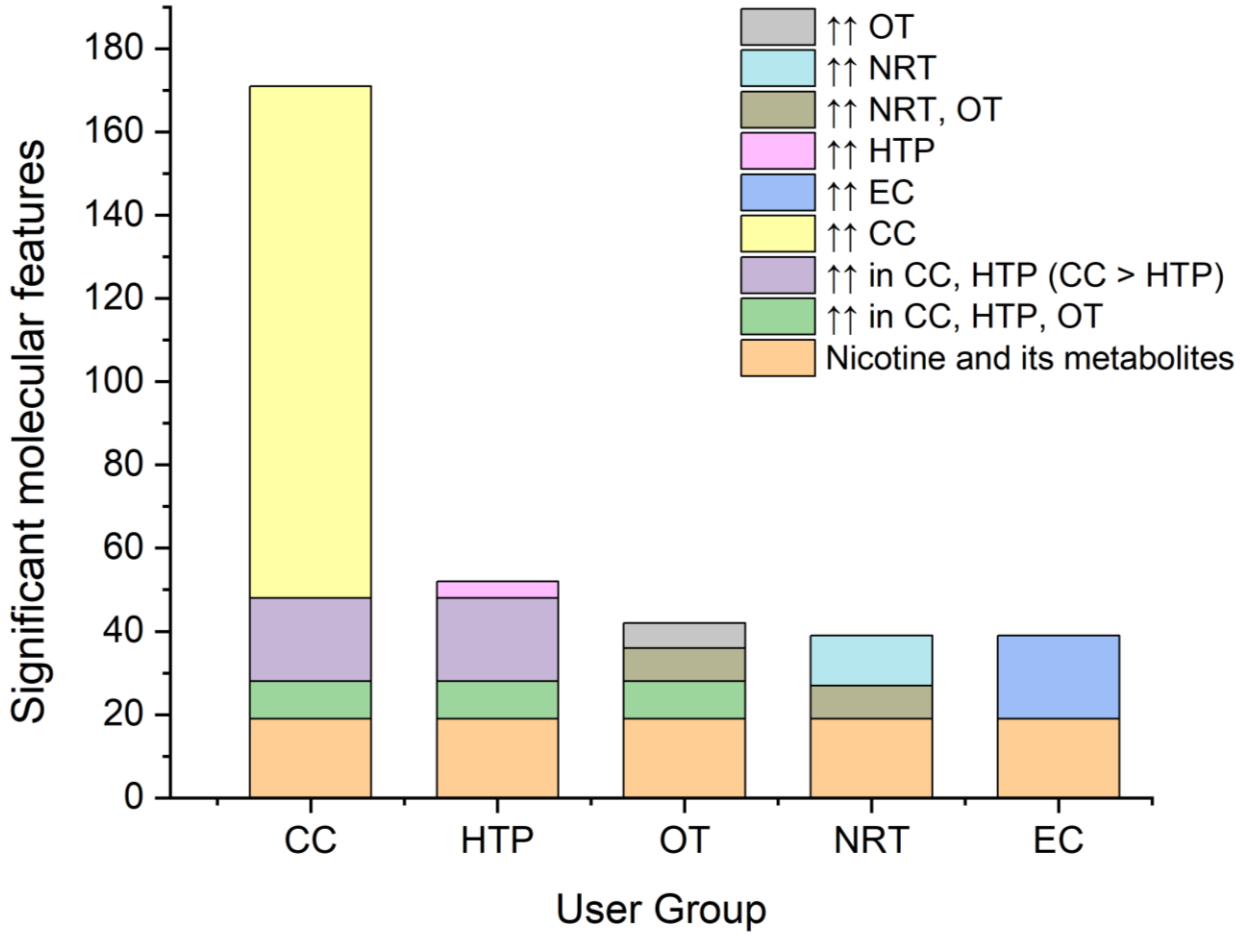
Statistical analysis: multi-group comparison:
$p < 0.05$; fold change (FC) > 2

Metabolite identification:

- Spectral information (exact mass / fragmentation / retention time)
- 3 Levels of confirmation (Level 1 > Level 2 > Level 3):
  - Level 1: Reference standard
  - Level 2: Spectral library
  - Level 3: In-Silico Fragmentation

Urinary Exposome

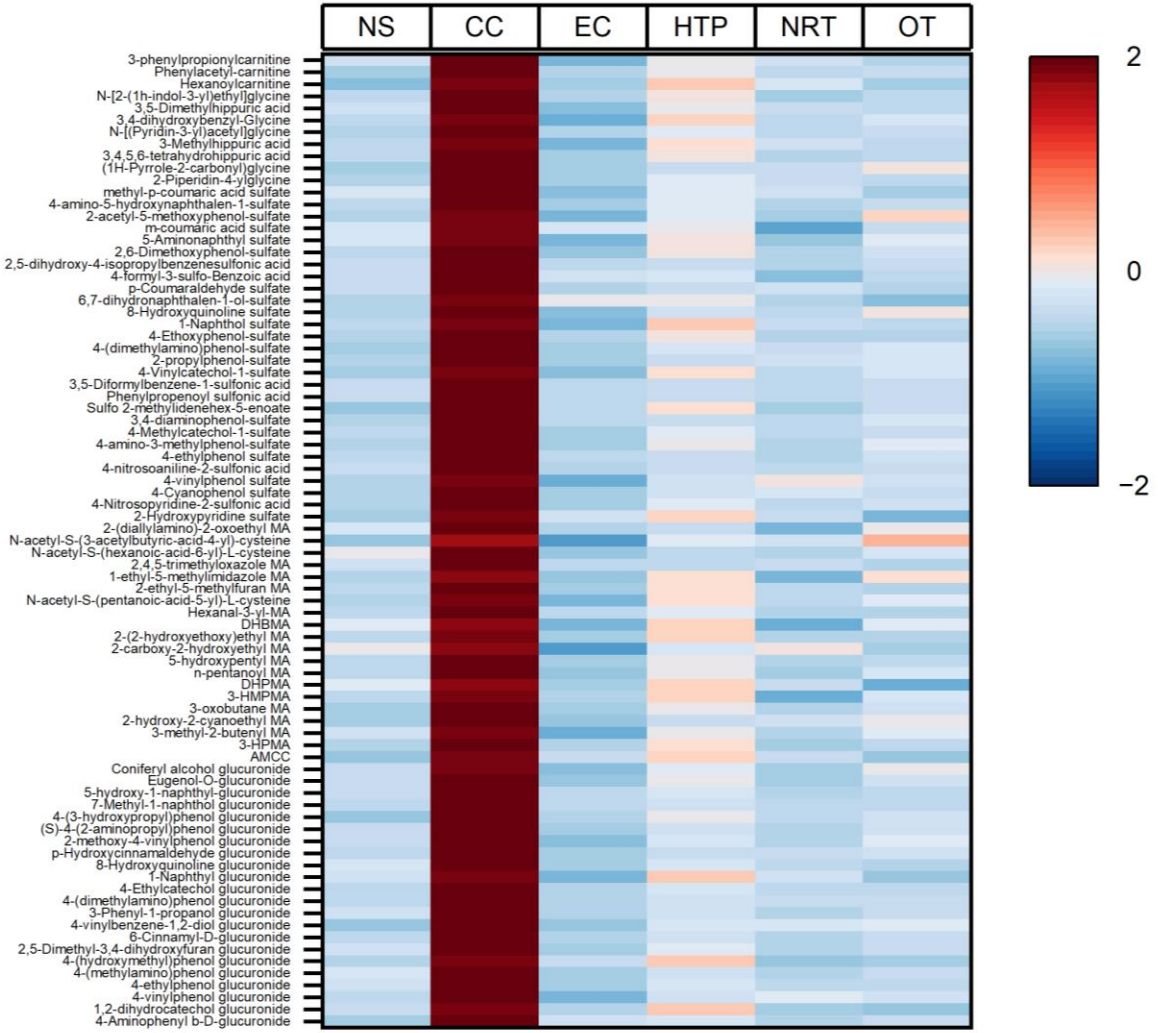
- A total of 208 molecular features specific to one nicotine product user group vs. NU (↑)
- Nicotine+metabolites identifed for all nicotine use groups (proof-of-concept 👍)
- Majority of elevated features in CC vs NU
- Specific features found for CC (123), NRT (12), EC (7), OT (6), HTP (4)
- Some features elevated in more than one group (e.g., NRT+OT; CC+HTP)



BoE specific to

CC

Biomarkers specific to CC users

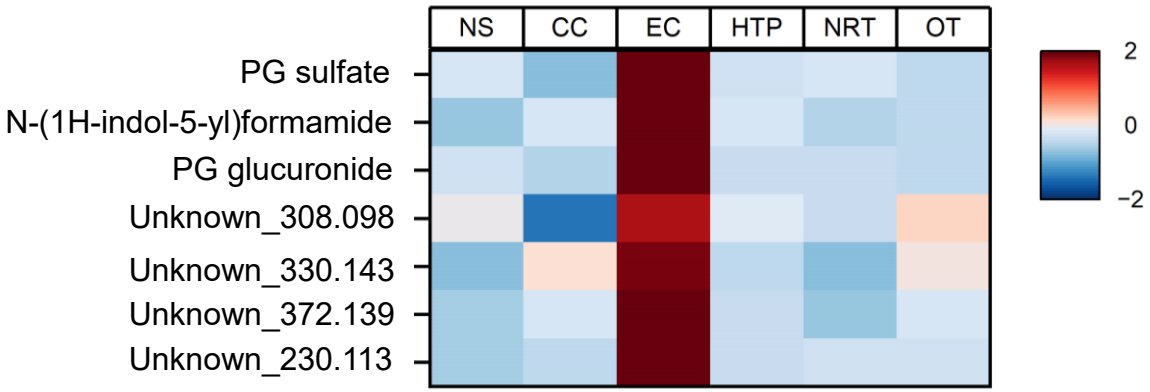


- Metabolites, especially Phase II conjugates, related to several chemical classes of toxicologically relevant compounds:
- VOCs: Aldehydes, Ketones, Epoxides, Alkenes, Alkanes
- Alcohols, phenols
- PAHs
- Aromatic amines
- Heterocyclic compounds
- Alkaloids

BoE specific to

EC

Biomarkers specific to EC users



Proposed Compound	Molecular Formula	MW	FC: EC/NS	Correlation with EC use (g liquid/d)
Propylene glycol (PG) sulfate	C3H8O5S	156.01	3.7	0.77
PG glucuronide	C9H16O8	252.09	7.5	0.82
N-(1H-indol-5-yl)formamide	C9H8N2O	160.06	4.5	0.66
Unknown_230.113	C7H14N6O3	230.11	5.0	0.77
Unknown_308.098	C12H18N2O6	308.10	3.4	0.57
Unknown_330.143	C14H22N2O7	330.14	13.7	0.35
Unknown_372.139	C13H20N6O7	372.14	25.5	0.66

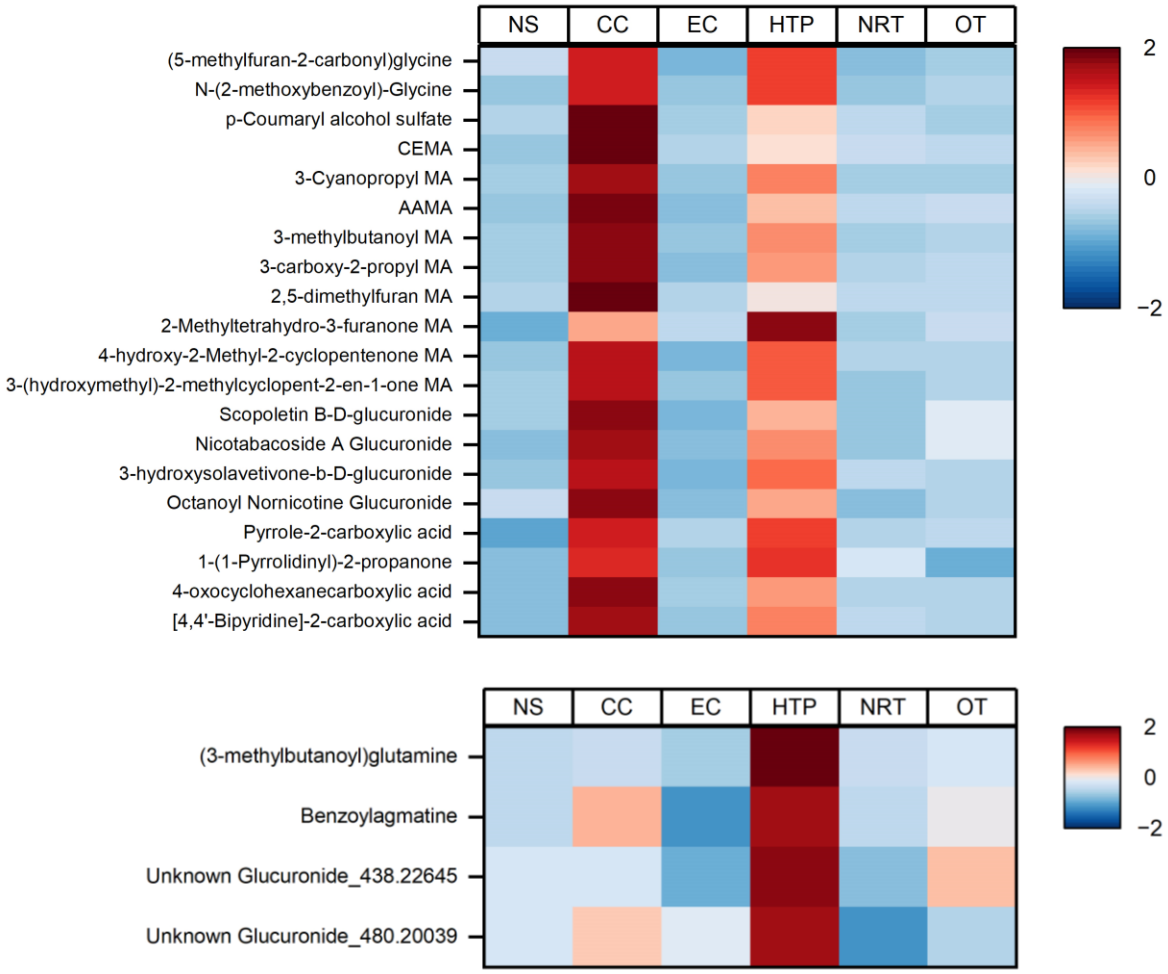
- PG conjugates elevated in EC users only
- Dose-response with EC use confirms specificity
- Results confirmed by targeted LC-MS/MS analysis of PG[1]

Burkhardt et al (2021): 1,2-Propylene Glycol: A Biomarker of Exposure Specific to e-Cigarette Consumption. *Separations*, 8, 180.

BoE specific to

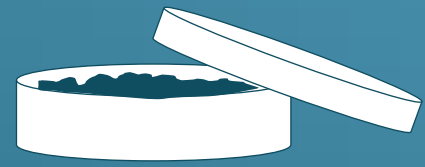
HTP

Biomarkers specific to HTP users

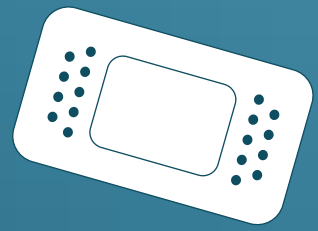


- Significantly different profile for HTP users vs CC users in Mercapturic acids and tobacco constituents
- Dose-response to CC/HTP use indicates specificity
- Results confirmed in targeted analysis (STPOST18)
- 4 BoE specific for HTP users, two not identified

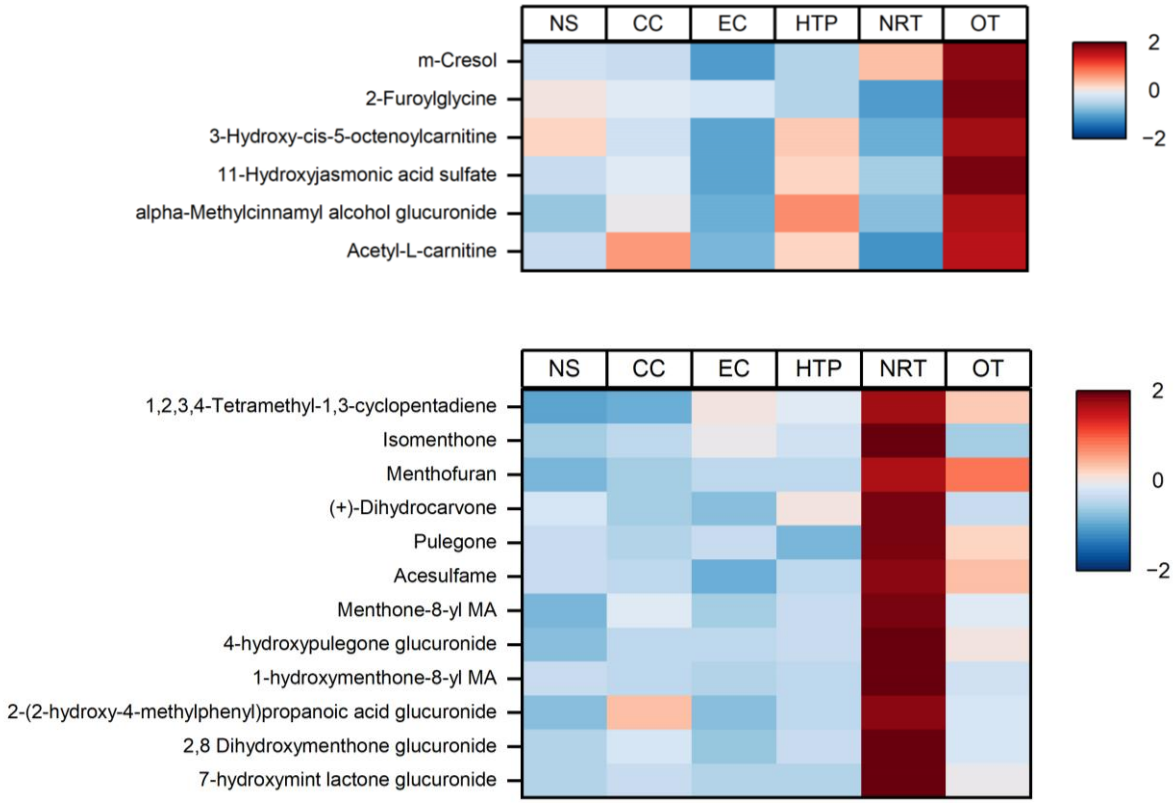
BoE specific to



OT & NRT



Biomarkers specific to OT & NRT users



- OT group consisted primarily of snus users
- 6 compounds elevated in OT group only

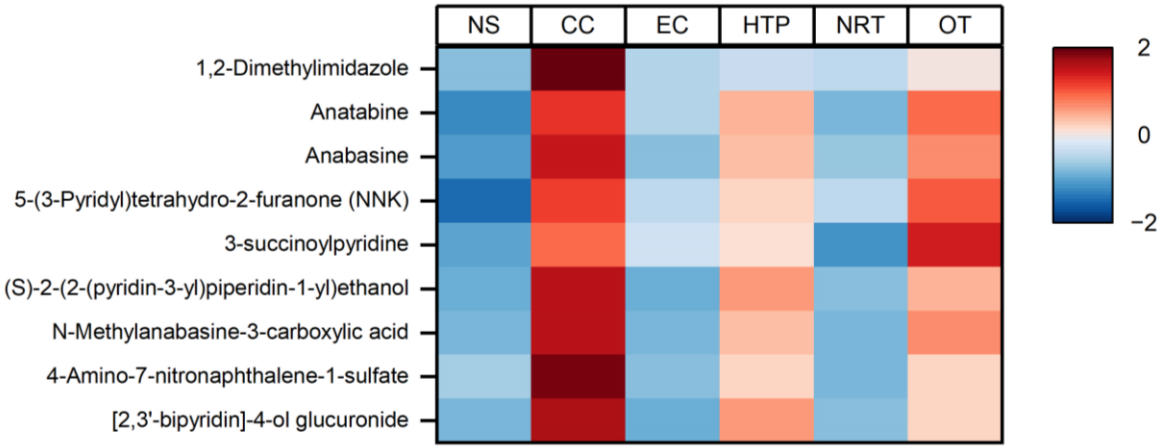
- NRT group consisted of users of nicotine gum and nicotine inhalers
- 12 compounds elevated in NRT group only

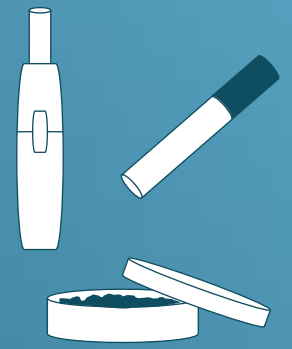
- Mostly monoterpenoid flavors and their metabolites for both groups, sweetener for NRT users

BoE specific to

tobacco-derived products

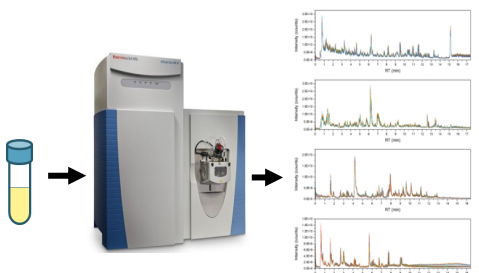
Biomarkers specific to tobacco-derived products

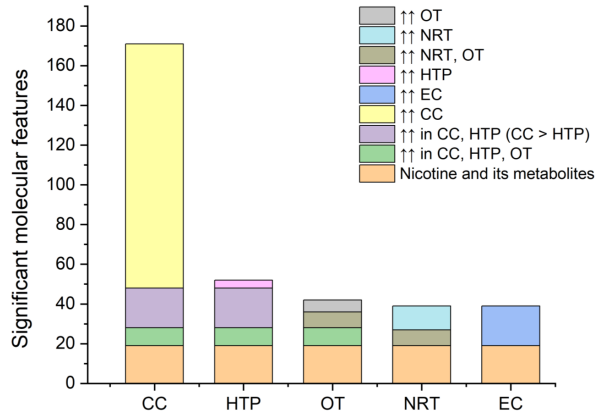


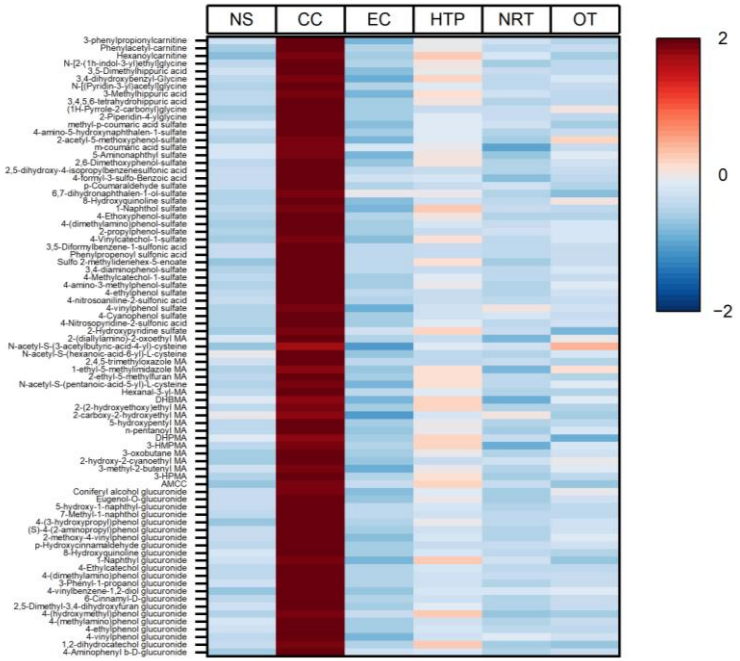


- 9 BoE identified as specific to tobacco derived product use of CC, HTP, OT
- Minor alkaloids anatabine/anabasine
- TSNAs
- Imidazole
- 2,3‘-Bipyridine

Conclusion

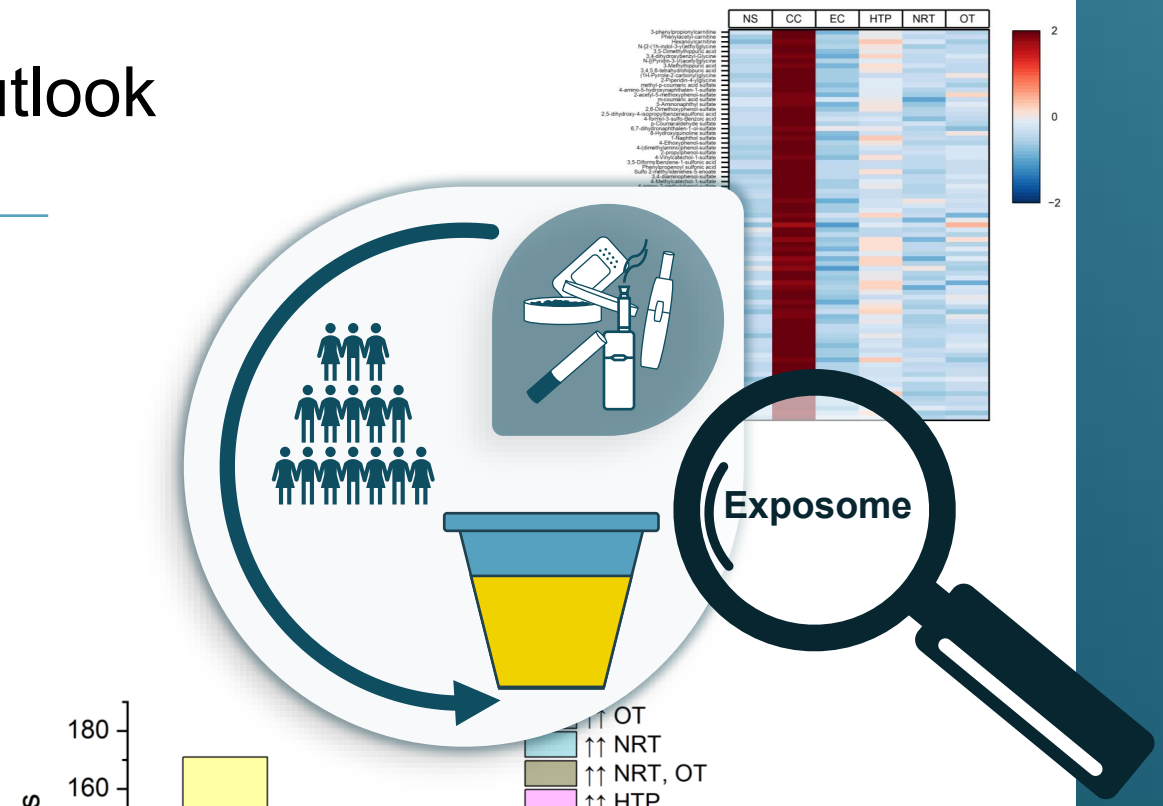


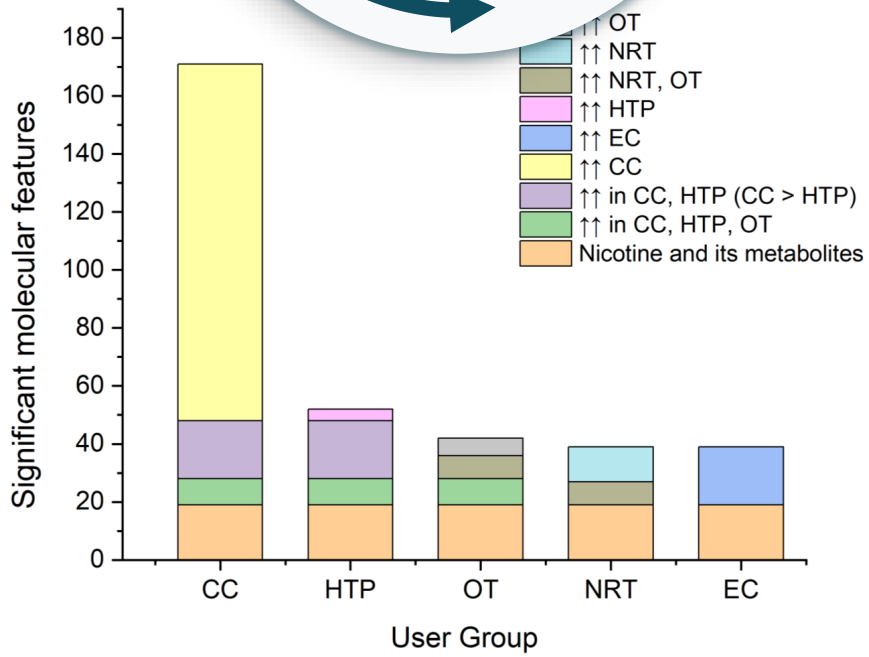




- Highly sensitive nontargeted method for urine analysis capable of detecting metabolites in the low ng/mL range
- **EC**: PG in urine as vaping-specific BoE
- **NRT / OT**: Unique flavor profile
- Panel of mercapturic acids potential to stratify **HTP** from other products including CC
- Minor alkaloids & TSNAs indicate use of tobacco-derived products

Outlook

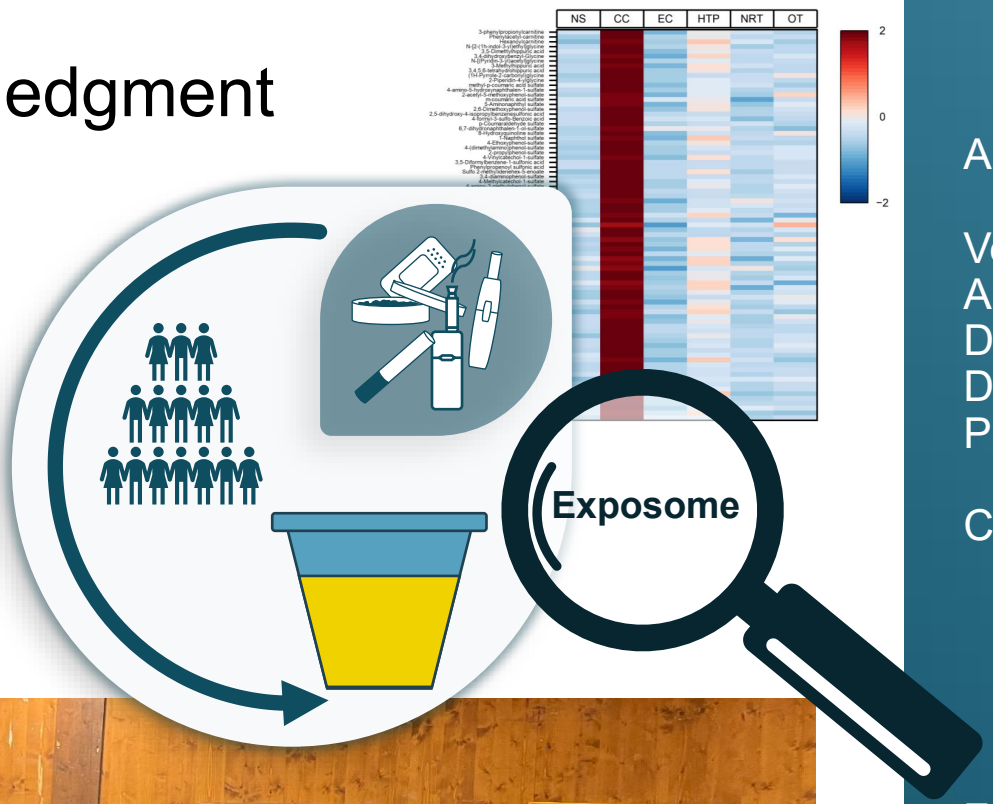




Specificity of the identified biomarkers needs confirmation by targeted analysis with larger sample sizes & in different populations

Implementation of specific BoE as a key tool for unambiguous verification of subjects' compliance
&
robust categorization of the products in the risk-continuum in future THR studies

Acknowledgment



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FOUNDATION FOR A SMOKE-FREE WORLD

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